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(54) Title: USE OF MONOCLONAL ANTIBODIES FOR DETERMINING SENSITIVITY TO ACETYLIZABLE DRUGS

(57) Abstract

Monoclonal antibodies having specific binding affinities for 1-methylxanthine and 5-amino-6-acetylamino-1-methyluracil and hybridoma cell lines producing the monoclonal antibodies. Immunoassay methods for measuring amounts of 5-amino-6-acetylamino-1-methyluracil and 1-methylxanthine in biological samples are described. An immunoassay method for determining an acetylator phenotype of an individual includes determining a ratio of acetylated metabolite to nonacetylated metabolite in a biological sample from the individual having been administered an acetylizable drug. The invention also provides an immunoassay method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug.

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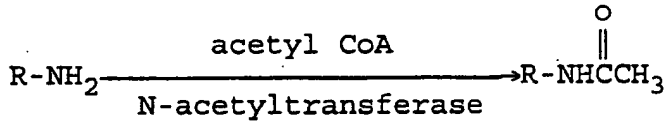
DESCRIPTION

USE OF MONOCLONAL ANTIBODIES FOR DETERMINING SENSITIVITY TO ACETYLIZABLE DRUGS

Research leading to the present invention was supported in part by the National Institute of Environmental Health Sciences (NIEHS 4-14346) and a National Research Service Award (651262-10). The U.S. government therefore has certain rights in the invention.

BACKGROUND OF THE INVENTION

Acetylation polymorphism refers to a genetically determined difference in the N-acetylation capacity of an individual for a variety of clinically useful drugs such as, for example, isoniazid (INH), procainamide (PA), hydralazine (HP), dapsone (DDS), sulfamethazine (SMZ), sulfasalazine, and amonafide as well as some putative carcinogenic arylamines. A general scheme of the N-acetylation reaction is described as follows:



Individuals are classified bimodally as either slow or rapid acetylators. According to family studies using INH, slow acetylators are autosomal homozygous recessive, while rapid acetylators are either heterozygous or homozygous dominants (Horai and Ishizaki (1987)). Presumably, the observed difference in the capacity of acetylation is a consequence of the liver content of N-acetyltransferase activity; in rapid acetylators it may be at least twice as high as in slow acetylators.

The frequency distribution of slow acetylators (Mendelian recessive traits) shows an interethnic difference that is highest in Orientals such as Japanese and Chinese, intermediate in Caucasian and African populations, and lowest in Egyptians. Thus, there are clinical implications not only for rational treatment with but also for developing side effect(s) related to acetylizable drugs in different racial groups.

Of particular clinical significance are relationships between variations in the metabolism of acetylizable drugs associated with such polymorphism and their toxic effects and the susceptibility of different phenotypes to disease. Because N-acetylation is an important pathway for metabolism of some drugs, and a large percentage of the population is missing this enzyme, it is important for physicians to be aware of the acetylation status of their patients. The general clinical consequences are that slow acetylators are more likely to develop adverse effect(s) of relevant drugs. Rapid acetylators are prone to show a less therapeutic response to the standard dose. One notable exception is the cancer drug, amonafide, which is more toxic in the acetylated form. The consequences of acetylation are known for many drugs and environmental contaminants, but are not known for others. A less costly and simpler assay would allow correlations to be drawn between environmental exposures and acetylation status.

For these reasons, acetylator phenotyping is desirable in order to properly adjust the dose, minimize possible adverse reactions, or otherwise increase the chance of therapeutic response before starting a patient on a long-term treatment. Potential clinically relevant toxicities and therapeutic responses to drugs

representing acetylation polymorphism include the following (Horai and Ishizaki (1987)):

INH- and HP-induced peripheral neuropathy. Peripheral neuropathy, developed with chronic administration of INH in patients with tuberculosis, is more common (20%) in slow acetylators than in rapid acetylators (3%); this toxicity is apparently a dose-related phenomenon.

INH-phenytoin interaction. Epileptic patients who had taken phenytoin for years rapidly developed central nervous system toxicity such as disorientation, ataxia and/or nystagmus when prophylactic antituberculosis therapy with INH was begun. Slow acetylators treated with INH are more susceptible to phenytoin toxicity because their higher concentration of INH may be more effectively related to the inhibition of the metabolism of phenytoin to its inactive hydroxylated metabolite.

HP-, PA- and INH- induced lupus erythematosus. HP, PA, and INH, each metabolized by N-acetylation, are known to cause drug-induced SLE.

Sulfasalazine-induced adverse reactions. Because sulfasalazine is metabolized initially to sulfapyridine and 5-aminosalicylic acid, and subsequently sulfapyridine is metabolized by N-acetylation, the adverse effects of sulfasalazine seem to depend largely upon the N-acetylation capacity of sulfapyridine. Total sulfapyridine concentration >50 μg/ml is

critical; a significant association of the occurrence of toxicity is at that level.

Arylamine-induced urinary bladder cancer. Carcinogenic arylamines such as ß-naphthylamine, 2-aminofluorene, benzidine, and 4-aminobiphenyl are possibly involved in the development of human urinary bladder cancer. Because N-acetylation is defined as a detoxication pathway of these arylamines, acetylation status could be associated with this carcinogenesis. The more invasive pathological forms of bladder cancers were found among slow acetylators. Arylamines, therefore, play a more crucial role in the development of bladder cancer in individuals with slow acetylation.

Amonafide-induced adverse reactions. Amonafide is a deoxyribonucleic acid intercalating agent with apparent antitumor activity in carcinoma of the breast and prostate. Rapid acetylators have a significantly greater toxicity than slow acetylators due to the production of the metabolite, N-acetyl-amonafide. Because of toxicity, patients undergoing therapy with this drug are now being phenotyped. (Ratain *et al.*, 1991).

The most widely used test drug for the assessment of acetylator phenotype was INH, because of the original interest and potential importance of phenotyping in patients with tuberculosis. Its complex metabolism and the need to determine its plasma half-life prevented INH from being a convenient method for routine phenotyping. Sulfamethazine has been an alternative for this purpose

because it is exclusively metabolized to acetylated SMZ, and both are measured with a simple photometric assay. Since approval of SMZ for human use has become difficult to obtain in the United States, the popularity of phenotyping with SMZ has decreased.

The use of two caffeine metabolite ratios for acetylator phenotyping was validated by Tang *et al.*, (1991) who demonstrated concordance with two sulfamethazine tests in 178 unrelated healthy subjects. The caffeine metabolites used for this purpose were 5-acetylamino-6-amino-3-methyluracil (AAMU), 1-methylxanthine (MX), and 1-methylurate (MU). The ratio AAMU/(AAMU + MX + MU), referred to as molar ratio of *N*-acetyltransferase, was compared with the ratio AAMU/MX. The results indicated that, for screening purposes, the acetylator phenotype can be determined by analysis of a 6-hour urine sample after a cup of coffee or strong tea or a can of caffeine-containing soft drink has been consumed. The ratio AAMU/MX is the ratio of choice for the study of subjects in whom variability of xanthine oxidase can be neglected; use of the ratio AAMU/(AAMU + MX + MU) appears appropriate for special purposes. Gender, ethnic origin, habitual or moderate consumption of coffee, tea, soft drinks, or ethanol, or cigarette smoking have little if any effect on the caffeine tests for acetylator phenotyping.

All assays in the literature to date use high pressure liquid chromatography (HPLC), gas chromatography (GC), or mass spectrometry (MS) for analysis of acetylator phenotype. These assays require the use of expensive equipment run by well trained personnel and are very time consuming. HPLC columns must be washed and regenerated and the use of peak height ratios instead of molar ratios increase day-to-day variability as a result of changing HPLC and instrument conditions.

The present invention provides a simple, cost- and time-efficient method for determining the acetylator phenotype of an individual taking advantage of the selectivity and sensitivity of an immunoassay. Using this method, large scale population studies can be done and correlated with environmental toxicity and drug exposure problems. This immunoassay could also be useful to the physician for predicting the drug metabolism phenotype of an individual who was going to require drug therapy. This invention therefore provides an immunoassay method for assessing drug metabolite ratios as a means of identifying those people in the population with fast and slow N-acetylation metabolizer phenotypes.

ABBREVIATIONS

AAMU	=	5-Amino-6-acetylamino-1-methyluracil
AFMU	=	5-Acetylamino-6-formylamino-3-methyluracil
BSA	=	Bovine serum albumin
DAMU	=	Diacetyl form of AAMU
DDS	=	Dapsone
EDC	=	1-Ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride
ELISA	=	Enzyme linked immunosorbent assay
HA	=	Hexanoic acid
HE	=	Hexanoic acid ethyl ester

	HP	=	Hydralazine
	HRP	=	Horseradish peroxidase
	INH	=	Isoniazid
	KLH	=	Keyhole Limpet Hemocyanin
	MIX	=	Acetic acid:methylene chloride:chloroform:water (100:60:90:50)
	MU or 1MU	=	1-Methyluric acid
	MX or 1MX	=	1-Methylxanthine
	ODP	=	O-Phenylenediamine dihydrochloride
	PA	=	Procainamide
	PBS	=	Phosphate buffered saline (0.05 M sodium phosphate, 0.15 M sodium chloride, pH 7.1)
	PLL	=	Poly-L-Lysine
	SMZ	=	Sulfamethazine
	137MX	=	1,3,7-Trimethylxanthine (caffeine)
	17MX	=	1,7-Methylxanthine
	17MU	=	1,7-Methyluracil

SUMMARY OF THE INVENTION

The present invention provides a monoclonal antibody having a specific binding affinity for 1-methylxanthine and without significant binding affinity for 5-amino-6-acetylamino-1-methyluracil and a hybridoma cell line producing said monoclonal antibody. "Without significant binding affinity" means that under the conditions in which the antibody binding to 1-methylxanthine is measured, less than about 5% binding of 5-amino-6-acetylamino-1-methyluracil is observed. The cell line is a hybrid of a spleen cell from a mouse immunized with a 1-methylxanthine-carrier conjugate and a mouse myeloma cell.

The present invention also provides a monoclonal antibody having a specific binding affinity for 5-amino-6-acetylamino-1-methyluracil and without significant binding affinity for 1-methylxanthine and a hybridoma cell line producing said monoclonal antibody. "Without significant binding affinity" means that under the conditions in which the antibody binding to 5-amino-6-acetylamino-1-methyluracil is measured, less than about 3% binding of 1-methylxanthine is observed. The cell line is a hybrid of a spleen cell from a mouse immunized with a 5-amino-6-acetylamino-1-methyluracil-carrier conjugate and a mouse myeloma cell.

Another aspect of the present invention is an immunoassay method for measuring amounts of 5-amino-6-acetylamino-1-methyluracil in a biological sample. In a general sense, the method comprises the steps of: i) reacting the biological sample with a monoclonal antibody having a specific binding affinity for 5-amino-6-acetylamino-1-methyluracil, and ii) measuring the amount of binding of the monoclonal antibody to 5-amino-6-acetylamino-1-methyluracil.

An immunoassay method for measuring amounts of 1-methylxanthine in a biological sample is also an aspect of the present invention. The method comprises: i) reacting the biological sample with a monoclonal antibody having a specific binding affinity for 1-methylxanthine, and ii) measuring the amount of binding of the monoclonal antibody to 1-methylxanthine.

A particular embodiment of the present invention is an immunoassay method for determining an acetylator phenotype of an individual using an acetylizable drug. An acetylator phenotype is the *in vivo* capacity of an individual to carry out N-acetylation. The method comprises the steps of: i) obtaining a first antibody having specific binding affinity for an acetylated metabolite of the acetylizable drug, ii) obtaining a second antibody having specific binding affinity for a nonacetylated metabolite of the acetylizable drug, iii) obtaining a biological sample from an individual having been administered the acetylizable drug, iv) quantitating the acetylated metabolite of the acetylizable drug in the biological sample, using the first antibody, v) quantitating the nonacetylated metabolite of the acetylizable drug in the biological sample, using the second antibody, and vi) determining a ratio of acetylated metabolite to nonacetylated metabolite. A fast acetylator phenotype of an individual has a greater ratio of acetylated metabolite to nonacetylated metabolite.

The acetylizable drug is a drug that is derivatized with an acetyl group during metabolism by the individual and may be a methylxanthine selected from the group consisting of 1,3,7-trimethylxanthine (caffeine), 1,3-dimethylxanthine (theophylline) and 3,7-dimethylxanthine (theobromine). The acetylizable drug may be administered orally in an amount sufficient to produce an acetylated

metabolite and a nonacetylated metabolite in the biological sample of the individual. The nonacetylated metabolite may be the acetylizable drug itself if it is excreted unmetabolized. The acetylated metabolite may be 5-amino-6-acetylamino-1-methyluracil and the nonacetylated metabolite may be 1-methylxanthine. The biological sample may be urine, blood or saliva and, in particular, the biological sample is urine.

In a general sense, a further aspect of the invention is a method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual. The method comprises the steps of: i) obtaining a first antibody having specific binding affinity for an acetylated metabolite of a methylxanthine, ii) obtaining a second antibody having specific binding affinity for a nonacetylated metabolite of the methylxanthine, iii) obtaining a biological sample from an individual having been administered the methylxanthine, iv) quantitating the acetylated metabolite of the methylxanthine in the biological sample, using the first antibody, v) quantitating the nonacetylated metabolite of the methylxanthine in the biological sample using the second antibody, and vi) determining a ratio of acetylated metabolite to nonacetylated metabolite. An individual's degree of sensitivity to an acetylizable therapeutic drug is correlated with the ratio of acetylated metabolite to nonacetylated metabolite depending upon whether the acetylated metabolite is the more toxic product or the less toxic product relative to the therapeutic drug.

The acetylizable control drug may be a methylxanthine selected from the group consisting of 1,3,7-trimethylxanthine, 1,3-dimethylxanthine and 3,7-dimethylxanthine and is administered orally in an amount sufficient to produce an acetylated metabolite and a

nonacetylated metabolite in the biological sample of the individual.

The acetylizable therapeutic drug may be selected from the group consisting of isoniazid, procainamide, hydralazine, dapsone, sulfamethazine, sulfasalazine and amonafide. With some therapeutic drugs, acetylation decreases the effectiveness of the drugs; with other therapeutic drugs, acetylation may increase the effectiveness undesirably. A fast acetylator, therefore, may not receive the dose needed or may suffer undue effects, whereas a slow acetylator may experience toxicity because the drug is not metabolized fast enough or may not receive the benefit if the acetylated form is the therapeutic form.

In the above methods, the quantitating steps may employ radioimmunoassays, enzyme immunoassays, or fluorescent immunoassays; the first and second antibodies may be monoclonal, and the quantitating steps include the steps of i) reacting the biological sample with the antibody, and ii) detecting binding of the antibody to the biological sample.

A further embodiment of the present invention is a method for assessing risk of toxicity to an acetylizable therapeutic drug in an individual. The method comprises the use of a first antibody having specific binding affinity for an acetylated metabolite of an acetylizable control drug and a second antibody having specific binding affinity for a nonacetylated metabolite of the acetylizable control drug to determine a ratio of acetylated metabolite to nonacetylated metabolite. An individual's risk of toxicity to an acetylizable therapeutic drug correlates with the ratio depending upon the particular drug. The drug toxicity may be drug related lupus erythematosus, isoniazid induced

neurotoxicity, phenytoin-isoniazid interactions, arylamine induced bladder cancer, or amonafide cancer therapy induced toxic reactions.

A further embodiment of the present invention is a kit useful for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual. The kit may comprise: i) carrier being compartmentalized to receive one or more container means in close confinement therein, ii) a first container means comprising a monoclonal antibody having specific binding affinity for 5-amino-6-acetylamino-1-methyluracil, and iii) a second container means comprising a monoclonal antibody having specific binding affinity for 1-methylxanthine. The antibody in the first container means and the antibody in the second container means may be immobilized on the container means.

A further aspect of the present invention is a process of preparing AAMU. The process comprises refluxing 1-methyluric acid with acetic acid anhydride in a mutual solvent to form a mixture of the mono- and diacetyl product, and reacting the mixture with a base to convert the mixture to the monoacetyl product, AAMU. The compositions, AAMU conjugated to KLH and AAMU conjugated to BSA are further embodiments of the present invention, as is a process of preparing AAMU conjugated to a carrier comprising the mixing of AAMU and the carrier in the presence of a coupling reagent. The carrier may be keyhole limpet hemocyanin or bovine serum albumin.

No one before the present inventor constructed an immunoassay for determining acetylator phenotype, in part, because the project required an unusual combination of interests: organic synthesis and purification, protein techniques including reaction and purification, and hybridoma technology which includes immunoassay and

cell culture, all combined with an interest in drug metabolism.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the presumed pathway for the metabolism of caffeine showing the relevant metabolites.

Fig. 2A shows the scheme for the preparation of modified 1MX for attachment to KLH and BSA. 1MX and 6-bromohexanoic acid (HA) ethyl ester were reacted and extracted with methylene chloride. The ester then was saponified and the resulting MXHA purified. Fig. 2B shows the scheme for the preparation of AAMU from 1-methyluric acid (1MU) by the reaction of 1MU with acetic acid, acetic anhydride, and pyridine to make a mixture of the mono- and diacetyl product. The mixture is then treated with ammonium hydroxide to produce primarily the mono-acetylated product.

Fig. 3 shows the U.V. spectra of: A. 1MX and 1MX modified by the addition of hexanoic acid; B. 1-methyluric acid starting material and the product AAMU.

Fig. 4 shows mass spectra (DCl + ammonia) demonstrating: A. the 1MX-HA conjugate (MW 280 + H^+); B. the mixture of the monoacetylated (MW 198 + H^+) and diacetylated (MW 240 + H^+) products.

Fig. 5 shows thin layer chromatography of: A. 1-methylxanthine, 1-MX reacted with 6-bromohexanoic acid ethyl ester, and the conjugate after saponification to remove the ethyl group; TLC on silica gel plate in chloroform: methanol (9:1). B. the preparation of AAMU from 1-methyluric acid (1MU) with a trace amount of the diacetyl product (DAMU); TLC on silica gel plate in the

lower phase of chloroform: methylene chloride: acetic acid: water (90:60:100:50).

Fig. 6A shows native BSA mixed with ^{14}C-AAMU and passed over a G-25 column for separation by molecular weight. Fig. 6B shows BSA conjugated to ^{14}C-AAMU with glutaraldehyde and passed over a G-25 molecular weight column.

Fig. 7 shows dilutions of antibodies obtained from hybridoma cell media and detected with goat anti-mouse-horseradish peroxidase (HRP) with ODP as the color reagent on ELISA plates. A. AAMU antibodies on 0.4 μg/well of BSA-AAMU, B. MX antibodies partially purified on 2.0 μg/well of PLL-MX.

Fig. 8 shows competition assays using theophylline as the competing hapten on two plates, one coated with PLL-theophylline and one with PLL-MX.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides monoclonal antibodies to metabolites of drugs that are representative of specific drug metabolism polymorphisms. These are used to measure urine concentrations of these metabolites to give a metabolic ratio descriptive of the phenotype. The N-acetylation system has been chosen as a model, with metabolites of caffeine as the haptens for monoclonal antibody development.

The present invention also provides a simple, cost-effective, and time-efficient immunoassay for the metabolites of caffeine, 5-amino-6-acetylamino-1-methyluracil (AAMU) and 1-methylxanthine (MX), to assess an individual's acetylator phenotype using urine. **Example 1** describes the synthesis of the caffeine

metabolites and linkage to proteins for immunization and ELISA plate coating; **Example 2** describes the immunization of animals to develop monoclonal antibodies to the metabolites; and **Example 3** describes the development of assays to utilize these antibodies in the quantification of the metabolites in urine. The immunoassay should be invaluable to clinicians for phenotyping individuals before drug therapy and to epidemiologists for large scale studies to correlate drug metabolism enzyme status with risk for cancer and disease.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Unless mentioned otherwise, the techniques employed herein are standard methodologies well known to one of ordinary skill in the art.

EXAMPLE 1

Synthesis of the Caffeine Metabolites AAMU and MX and Linkage to Carrier Proteins

The initial step involved preparing protein conjugates of the two metabolites, 5-acetylamino-6-amino-3-methyluracil (AAMU) and 1-methylxanthine (1MX) for specific antibody production to the two metabolites exclusive of each other.

Methods

AAMU preparation - The method of Khmelevskii *et al.*, (1958), was adapted, using 1-methyluric acid (1MU) as the starting material. 1MU (0.25 g) was added to 1.1 ml of a

solvent containing acetic acid anhydride: glacial acetic acid: pyridine (15:18:10). The solution was stirred and refluxed in a small flask heated in an oil bath at 150°C for 1.5 hours or until clarified. Water (0.1 ml) was added and refluxed for a further 30 minutes. The solution was cooled and filtered. The filtrate was diluted with water and methanol, rotoevaporated to remove the pyridine, and then freeze dried. The resulting residue was a mixture of the mono- and diacetyl product. The solid (0.15 g) was dissolved in a minimum amount of warm water (0.8 ml) and 2.5 N ammonium hydroxide (0.4 ml). The solution was heated for 1 hour at 100°C, cooled, neutralized, and then centrifuged. The resulting precipitate containing the monoacetyl derivative was washed in cold water and dried. The supernatant fractions containing a mixture of mono- and diacetyl products were also dried. Products were monitored using silica gel thin layer chromatography (TLC) with a mobile phase consisting of acetic acid: methylene chloride: chloroform: water (lower phase) 100:60:90:50 (Tang *et al.*, 1983) (MIX).

MX-hexanoic acid (MXHA) prep - Addition of a six carbon linker to MX was accomplished by a modification of methods described by Cooke, *et al.*, (1976). The original method was developed for theophylline and used 6-bromohexanoic acid lithium salt. The present method used 1-methylxanthine (100 mg, Sigma) and 6-bromohexanoic acid ethyl ester (BrHAEE) (0.107 ml, Sigma). All ingredients and the reaction vessel were dried overnight. MX in 1.8 ml of N,N-dimethylformamide and 0.62 mmoles of potassium carbonate (85.7 mg) were incubated at 25-30°C with BrHAEE for 4 hours. Water (2.2 ml) was added to stop the reaction. After cooling, the reaction mixture was extracted one time with 10 ml methylene chloride (MC) which was dried under nitrogen. (Unreacted MX (approximately 70%) can be recovered by precipitation

with acid from the water layer.) The dried MC extract was dissolved in 2 ml 1 M NaOH and warmed at 37°C for 2 hours to saponify the ester. MC was used to extract unreacted material. The MX-hexanoic acid (MX-HA) was then precipitated with HCl. Silica gel TLC using chloroform:methanol (9:1) as running solvent was used to monitor the reactions.

KLH-AAMU conjugate - KLH (Imject Keyhole Limpet Hemocyanin - (Pierce) in .083M phosphate, 0.9M NaCl, pH 7.2 and stabilizers) was diluted to 10 mg/ml in 2 ml water and sampled for UV and protein determination. Citraconic anhydride (Sigma) was diluted 1 to 100 and 2 ml were added slowly to KLH, while using 0.15 N NaOH to keep the pH 8-9. The solution was stirred for 1 hour in order to block free amine groups (Harlow and Lane, 1988). After overnight dialysis, 10 mg/ml 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC, Pierce Biochemicals) was added, maintaining pH at 8-9 for 10 minutes. Three milligrams of AAMU were added and stirred for 5 hours, maintaining pH. The solution was dialyzed overnight. The loss of free hapten was monitored by A_{264}. The amount of addition of AAMU to KLH was estimated by the change in absorbance at 264nm using an extinction coefficient of 11000 M^{-1}. Conjugation added approximately 2 nmoles AAMU/mg KLH.

BSA-AAMU conjugate - AAMU (36 μmoles) was added to 25 mg of bovine serum albumin (BSA) (0.36 μmoles) in 5 ml of PBS. Glutaraldehyde (40 μmoles) in 2 ml of water was added slowly, stirring for 1 hour. The reaction was stopped with 1 M glycine (2 ml) for one hour before dialyzing overnight with 3 changes of PBS. Conjugation added from 4-10 moles AAMU/mole BSA.

BSA- poly-l-lysine (PLL)- and KLH-1MX preparation - 1MXHA (1.5-2.0 mg) was dissolved in 10 ml water with a

minimum amount of NaOH. EDC (5 mg/ml) was added and after 5 minutes, protein (10-15 mg in 1-3 ml water) was added and incubated at room temperature for 2 hours. Sodium acetate, pH 4.2 (1M) was added to 0.1 M to stop the reaction, and the mixture was dialyzed overnight with three changes of PBS.

TNBS Assay - The TNBS assay (Fields, 1972) utilizing 2,4,6-trinitrobenzene sulfonic acid (Sigma, 100mg/0.2ml) to measure free lysine groups was used to monitor the addition of hapten to protein when lysine groups were involved. Absorbance at 420 nm with an extinction coefficient of $19200M^{-1}$ cm^{-1} for ϵ-amino groups was used to estimate the number of lysines reacted.

Determination of extinction coefficients - Extinction coefficients for 1 MXHA were determined on 0.01 and 0.001 mg/ml solutions at neutral and basic pH at A_{270} and A_{290} respectively to give an E of 5000 $M^{-1}cm^{-1}$ at both absorbances. The extinction coefficient for AAMU was determined under neutral conditions on solutions from 1 to 100 μM at A_{264} to give a value of 10000 $M^{-1}cm^{-1}$.

Biotinylation of AAMU and/or protein-AAMU - Immunopure NHS-LC-Biotin (Pierce, 7.0 mg/ml) was attached to AAMU (0.5 mg/ml) by reacting in 50 mM $NaCO_3$ buffer, pH 8.5 for 30 minutes at room temperature. The reaction was monitored by TLC using the MIX from above on silica plates. Immunopure NHS-LC Biotin (1 mg/ml) was attached to protein-AAMU (1 mg/ml) by reacting in 50mM $NaCO_3$ buffer, pH 8.5 for 1 hour at room temperature. The reaction mix was then passed over a G-25 molecular sieve column to separate the biotin and the protein fractions.

EXAMPLE 2

Monoclonal Antibodies to the Metabolites

Methods

Monoclonal antibody formation - Eight week old female Balb/c mice were injected with antigen conjugated to KLH (25 μg/mouse) in Freund's complete adjuvant (Bjercke *et al.*, 1986; Kennette *et al.*, 1978). The mice were reinjected at two to three week intervals in Freund's incomplete adjuvant at least twice. When the mouse serum gave a positive titer, animals were hyperboosted with 25 μg/day for three days without adjuvant before removing the spleen. Hybridoma formation followed the method of Bjercke *et al.*, (1986). Screening for positive clones was accomplished using BSA conjugate (0.4-2.0 μg/well protein) on an ELISA plate. Because of instability of the clones, fresh media was needed during the hybridoma formation. At all times media was less than 1 month old. Colonies were allowed to develop in 24 well plates and then cloned when activity occurred. Many clones lost activity over time.

Results

Approximately 7000 colonies were screened for AAMU antibody activity and 4000 for MX antibody activity. From these, six AAMU and 1 MX colonies were stable and non cross-reactive with the other metabolite. The hybridoma cell lines were tested on plates containing only the blocking agent, BSA alone, or BSA-AAMU (1 μg/well). These colonies also tested negative or very low versus PLL-MX (5μg/well). Table 1 describes hybridoma cell lines producing antibodies to AAMU.

TABLE 1

Hybridoma Cell Lines Producing Antibodies to AAMU[1].

Hybridoma	Isotype	BSA-AAMU	BSA	Blank
O1D2E3	IgM K	23.2±2.5	1.5±0.2	1.0±0.5
O1D2F3	IgM K	27.4±1.3	2.6±1.4	1.0±0.5
O1D4E2	IgM K	23.0±2.3	1.6±0.1	1.2±0.2
O2A1C11	IgM K	20.8±0.3	1.4±0.8	0.8±0.2
O3D6F9	IgM K	29.4±1.2	3.5±1.5	1.4±0.1
O3C6C4	IgM K	22.1±2.4	2.6±0.2	0.8±0.3

1. OD_{492} per 100 min. per well

The clone 10BB3 produces an IgG antibody to MX that cross-reacts with theophylline but less than about 5% with AAMU.

EXAMPLE 3

Determination of Acetylator Phenotype

The N-acetylation phenotyping is performed in the following manner. Individuals to be tested will be administered the desired amount of a methylxanthine, either in coffee, tea, cola or by tablet. The methylxanthine may be caffeine, theophylline or theobromine. The amount to be administered is 100-200 mg or an amount sufficient to produce acetylated and nonacetylated metabolites in the biological sample to be collected. A small sample of urine will be collected after consumption of the methylxanthine. A 4-5 hour interval between the consumption of the methylxanthine and the collection of urine is an optimal interval. However, the length of time is not fixed at 4-5 hours for the practice of the invention. Sodium hydroxide may be added to the urine sample to convert AFMU to AAMU, then the sample is neutralized with acid and used immediately or frozen. The neutralized urine sample will be used

directly, if possible, in a competition assay. If urinary components are found to interfere in the assay, a simple organic extraction will be performed to obtain the metabolites. The MX concentration will be determined by coating an ELISA plate with PLL-MX. Antibody in the presence of various levels of MX will be used as the standard. The urine samples will then be used in the presence of antibody and the concentration of MX calculated from the standard curve. A second antibody will be a goat-anti-mouse-HRP conjugate, followed by ODP as the color reagent. The OD_{492} is measured to quantitate the antibody-antigen reaction and the result compared to a standard curve.

As an example, a competition assay for the hapten, theophylline is as follows: - Immulon 3 (Dynatek) plates were coated with PLL-theo (1μg/well by weight) overnight in borate coating buffer followed by washing three times in Tween-saline (TS, 0.05% Tween 80, 0.15M sodium chloride). Reconstituted non-fat dry milk (1%) was used to block non specific binding for 1 hour. The plates were again washed three times Reconstituted non-fat dry milk (0.1%) in Tween-PBS (TPBS) was used for all dilutions. Anti-theophylline antibody was diluted to a final concentration of 1:128 in dilutions of theophylline of 4nM to 2μM in final concentration. Antibody and hapten were mixed for 30 minutes, and added to the plate for four hours. After three washes, the second antibody was added in milk buffer for 45 minutes. The plates were again washed three times. O-Phenylenediamine dihydrochloride (ODP, Pierce Biochemicals) was used in citrate-phosphate buffer at 0.2 mg/ml with H_2O_2 (50 μl of 3% in 20 ml) for 6 minutes. A similar assay may be used to determine the concentration of MX.

The method of assay for the AAMU may be carried out as follows. The IgM antibody may be attached to an ELISA

plate either directly or through a goat-anti-mouse-antibody. The AAMU will then be competed against AAMU attached either to biotin or to biotin attached to PLL or BSA. The detection will be accomplished with an avidin-HRP conjugate followed by ODP color reaction. When the concentration of both metabolites has been determined, a ratio of AAMU to MX will be used to determine the acetylator phenotype.

These antibodies can be used to measure metabolites in the blood, saliva or other bodily liquids with possible modification of the methods. For example, protein may need to be precipitated out of the sample for detection in bodily liquids other than urine.

These monoclonal antibody assays for the identification of the N-Acetylation polymorphism will have even broader applicability. Caffeine is used as a metabolic probe to screen for the activities of at least two other enzymes. Cytochrome P4501A2 activity can be estimated by the ratio (AAMU + MX + MU)/17MU and xanthine oxidase by the ratio MU/(MX + MU) (Kalow, W. *et al.*, 1991). One skilled in the art would see that the development of other antibodies, in conjunction with those already developed, would allow the identification of these metabolic ratios.

The following references are incorporated in pertinent part by reference herein for the reasons cited above.

REFERENCES

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It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

CLAIMS:

1. A monoclonal antibody having a specific binding affinity for 1-methylxanthine and without significant binding affinity for 5-amino-6-acetylamino-1-methyluracil.

2. A monoclonal antibody having a specific binding affinity for 5-amino-6-acetylamino-1-methyluracil and without significant binding affinity for 1-methylxanthine.

3. A hybridoma cell line producing monoclonal antibody having a specific binding affinity for 1-methylxanthine and without significant binding affinity for 5-amino-6-acetylamino-1-methyluracil.

4. The hybridoma cell line of claim 3 wherein the cell line is a hybrid of a spleen cell from a mouse immunized with a 1-methylxanthine-carrier conjugate and a mouse myeloma cell.

5. A hybridoma cell line producing monoclonal antibody having a specific binding affinity for 5-amino-6-acetylamino-1-methyluracil and without significant binding affinity for 1-methylxanthine.

6. The hybridoma cell line of claim 5 wherein the cell line is a hybrid of a spleen cell from a mouse immunized with a 5-amino-6-acetylamino-1-methyluracil-carrier conjugate and a mouse myeloma cell.

7. An immunoassay method for measuring amounts of 5-amino-6-acetylamino-1-methyluracil in a biological sample comprising:

reacting the biological sample with a monoclonal antibody having a specific binding affinity for 5-amino-6-acetylamino-1-methyluracil; and

measuring the amount of binding of the monoclonal antibody to 5-amino-6-acetylamino-1-methyluracil.

8. An immunoassay method for measuring amounts of 1-methylxanthine in a biological sample comprising:

reacting the biological sample with a monoclonal antibody having a specific binding affinity for 1-methylxanthine; and

measuring the amount of binding of the monoclonal antibody to 1-methylxanthine.

9. A method for determining an acetylator phenotype of an individual using an acetylizable drug, the method comprising:

obtaining a first antibody having specific binding affinity for an acetylated metabolite of the acetylizable drug;

obtaining a second antibody having specific binding affinity for a nonacetylated metabolite of the acetylizable drug;

obtaining a biological sample from an individual having been administered the acetylizable drug;

quantitating the acetylated metabolite of the acetylizable drug in the biological sample, using the first antibody;

quantitating the nonacetylated metabolite of the acetylizable drug in the biological sample, using the second antibody; and

determining a ratio of acetylated metabolite to nonacetylated metabolite;

wherein a fast acetylator phenotype of an individual has a greater ratio of acetylated metabolite to nonacetylated metabolite.

10. The method of claim 9 where an acetylator phenotype is the *in vivo* capacity of an individual to carry out N-acetylation.

11. The method of claim 9 wherein the acetylizable drug is a methylxanthine selected from the group consisting of 1,3,7-trimethylxanthine, 1,3-dimethylxanthine and 3,7-dimethylxanthine.

12. The method of claim 9 where the acetylizable drug is administered orally in an amount sufficient to produce an acetylated metabolite and a nonacetylated metabolite in the biological sample of the individual.

13. A method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual, the method comprising:

obtaining a first antibody having specific binding affinity for an acetylated metabolite of an acetylizable control drug;

obtaining a second antibody having specific binding affinity for a nonacetylated metabolite of the acetylizable control drug;

obtaining a biological sample from an individual having been administered the acetylizable control drug;

quantitating the acetylated metabolite of the control drug in the biological sample, using the first antibody;

quantitating the nonacetylated metabolite of the control drug in the biological sample using the second antibody; and

determining a ratio of acetylated metabolite to nonacetylated metabolite;

wherein an individual's degree of sensitivity to an acetylizable therapeutic drug is correlated to the ratio of acetylated metabolite to nonacetylated metabolite.

14. The method of claim 13 wherein the acetylizable control drug is a methylxanthine selected from the group consisting of 1,3,7-trimethylxanthine, 1,3-dimethylxanthine and 3,7-dimethylxanthine.

15. The method of claim 13 where the acetylizable control drug is administered orally in an amount sufficient to produce an acetylated metabolite and a nonacetylated metabolite in the biological sample of the individual.

16. A method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual, the method comprising:

obtaining a first antibody having specific binding affinity for an acetylated metabolite of a methylxanthine;

obtaining a second antibody having specific binding affinity for a nonacetylated metabolite of the methylxanthine;

obtaining a biological sample from an individual having been administered the methylxanthine;

quantitating the acetylated metabolite of the methylxanthine in the biological sample, using the first antibody;

quantitating the nonacetylated metabolite of the methylxanthine in the biological sample using the second antibody; and

determining a ratio of acetylated metabolite to nonacetylated metabolite;

wherein an individual's degree of sensitivity to an acetylizable therapeutic drug is correlated to the ratio of acetylated metabolite to nonacetylated metabolite.

17. The method of claim 13 or 16 wherein the acetylizable therapeutic drug is selected from the group consisting of isoniazid, procainamide, hydralazine, dapsone, sulfamethazine, sulfasalazine and amonafide.

18. The method of claim 9, 13 or 16 wherein the acetylated metabolite is 5-amino-6-acetylamino-1-methyluracil.

19. The method of claim 9, 13 or 16 wherein the nonacetylated metabolite is 1-methylxanthine.

20. The method of claim 16 where the methylxanthine is administered orally in an amount sufficient to produce an acetylated metabolite and a nonacetylated metabolite in the biological sample of the individual.

21. A method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual, the method comprising:

obtaining a first antibody having specific binding affinity for 5-amino-6-acetylamino-1-methyluracil;

obtaining a second antibody having specific binding affinity for 1-methylxanthine;

obtaining a biological sample from an individual having been administered a methylxanthine;

quantitating 5-amino-6-acetylamino-1-methyluracil in the biological sample using the first antibody;

quantitating 1-methylxanthine in the biological sample using the second antibody; and

determining a ratio of 5-amino-6-acetylamino-1-methyluracil to 1-methylxanthine;

wherein an individual's degree of sensitivity to an acetylizable therapeutic drug is correlated to the ratio of 5-amino-6-acetylamino-1-methyluracil to 1-methylxanthine.

22. The method of claim 21 where the methylxanthine is administered orally in an amount sufficient to produce 5-amino-6-acetylamino-1-methyluracil and 1-methylxanthine in the biological sample of the individual.

23. The method of claim 9, 13, 16 or 21 where the biological sample is urine, blood or saliva.

24. The method of claim 9, 13, 16 or 21 where the biological sample is urine.

25. A method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual, the method comprising:

obtaining a first antibody having specific binding affinity for 5-amino-6-acetylamino-1-methyluracil;

obtaining a second antibody having specific binding affinity for 1-methylxanthine;

obtaining a urine sample from an individual having been administered a methylxanthine;

quantitating 5-amino-6-acetylamino-1-methyluracil in the urine sample using the first antibody;

quantitating 1-methylxanthine in the urine sample using the second antibody; and

determining a ratio of 5-amino-6-acetylamino-1-methyluracil to 1-methylxanthine;

wherein an individual's degree of sensitivity to an acetylizable therapeutic drug is correlated to the ratio of 5-amino-6-acetylamino-1-methyluracil to 1-methylxanthine.

26. A method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual, the method comprising:

obtaining a first antibody having specific binding affinity for 5-amino-6-acetylamino-1-methyluracil;

obtaining a second antibody having specific binding affinity for 1-methylxanthine;

obtaining a urine sample from an individual having been administered caffeine;

quantitating 5-amino-6-acetylamino-1-methyluracil in the urine sample using the first antibody;

quantitating 1-methylxanthine in the urine sample using the second antibody; and

determining a ratio of 5-amino-6-acetylamino-1-methyluracil to 1-methylxanthine;

wherein an individual's degree of sensitivity to an acetylizable therapeutic drug is correlated to the ratio of 5-amino-6-acetylamino-1-methyluracil to 1-methylxanthine.

27. The method of claim 26 where the caffeine is administered orally in an amount sufficient to produce 5-amino-6-acetylamino-1-methyluracil and 1-methylxanthine in the urine sample of the individual.

28. The method of claim 9, 13, 16, 21, 25 or 26 wherein the quantitating steps employ radioimmunoassays, enzyme immunoassays, or fluorescent immunoassays.

29. The method of claim 9, 13, 16, 21, 25 or 26 where the first and second antibodies are monoclonal.

30. The method of claim 9, 13, 16, 21, 25 or 26 where the quantitating steps include the steps of

reacting the biological sample with the antibody ; and

detecting binding of the antibody to the biological sample.

31. A method for assessing risk of toxicity to an acetylizable therapeutic drug in an individual comprising the use of a first antibody having specific binding

affinity for an acetylated metabolite of an acetylizable control drug and a second antibody having specific binding affinity for a nonacetylated metabolite of the acetylizable control drug to determine a ratio of acetylated metabolite to nonacetylated metabolite wherein an individual's risk of toxicity to an acetylizable therapeutic drug is correlated to the ratio.

32. The method of claim 31 where the drug toxicity is drug related lupus erythematosus.

33. The method of claim 31 where the drug toxicity is isoniazid induced neurotoxicity.

34. The method of claim 31 where the drug toxicity is phenytoin-isoniazid interactions.

35. The method of claim 31 where the drug toxicity is arylamine induced bladder cancer.

36. The method of claim 31 where the drug toxicity is amonafide cancer therapy induced toxic reactions.

37. A kit useful for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual which comprises:

a carrier being compartmentalized to receive one or more container means in close confinement therein;

a first container means comprising a monoclonal antibody having specific binding affinity for 5-amino-6-acetylamino-1-methyluracil; and

a second container means comprising a monoclonal antibody having specific binding affinity for 1-methylxanthine.

38. The kit of claim 37 where the antibody in the first container means is immobilized on the container means.

39. The kit of claim 37 wherein the antibody in the second container means is immobilized on the container means.

40. A process of preparing AAMU comprising:

refluxing 1-methyluric acid with acetic acid anhydride in a mutual solvent to form a mixture of the mono- and diacetyl product; and

reacting the mixture with a base to convert the mixture to the monoacetyl product, AAMU.

41. AAMU conjugated to KLH.

42. AAMU conjugated to BSA.

43. A process of preparing AAMU conjugated to a carrier comprising the mixing of AAMU and the carrier in the presence of a coupling reagent.

44. The process of claim 4 where the carrier is keyhole limpet hemocyanin.

45. The process of claim 4 where the carrier is bovine serum albumin.

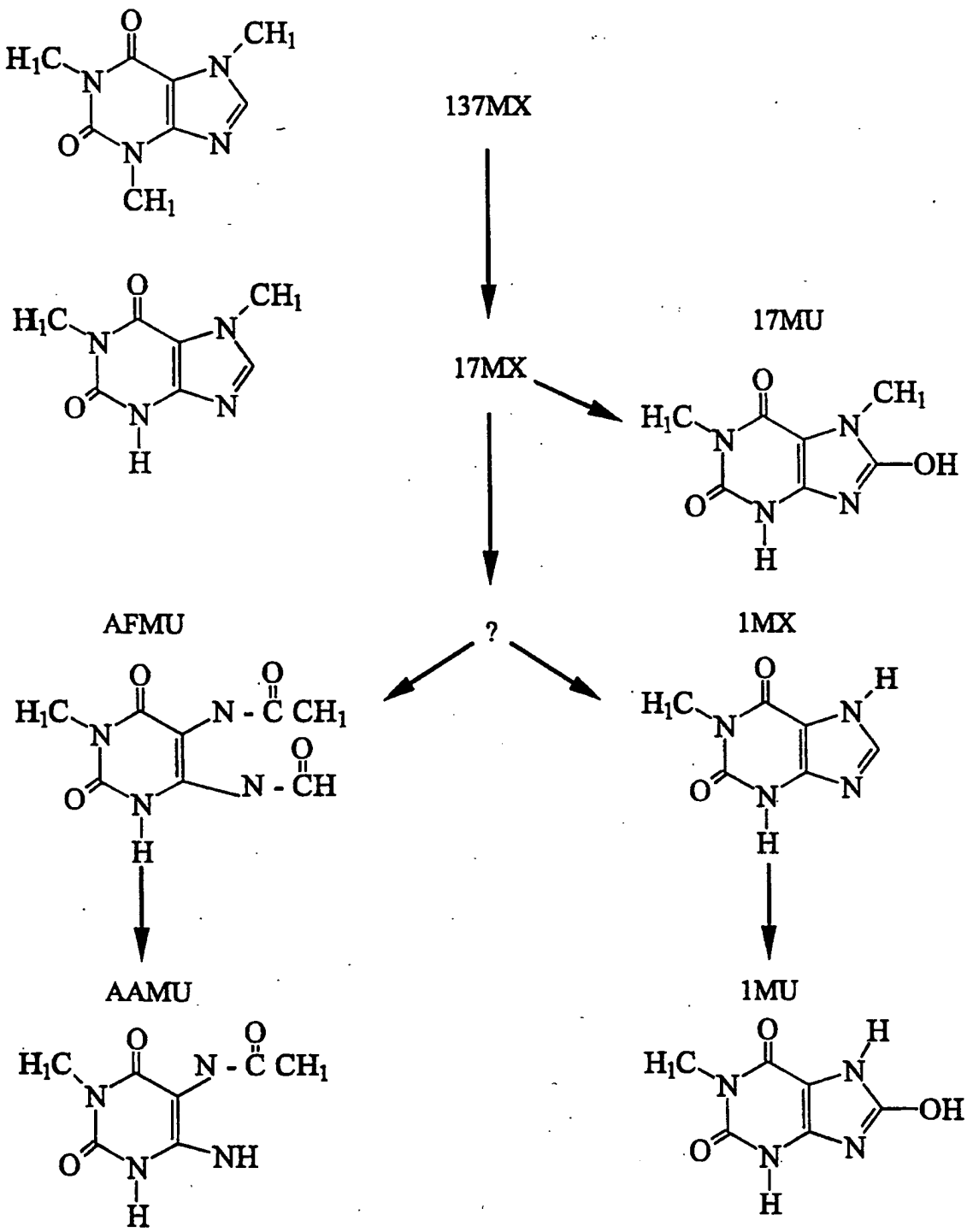
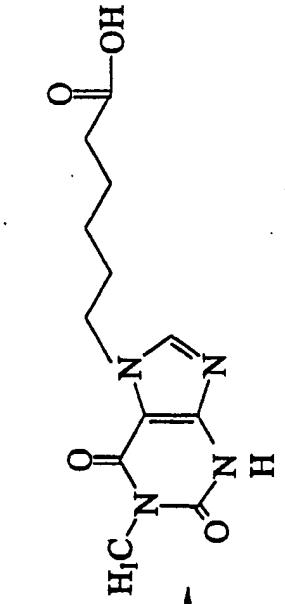


Fig. 1



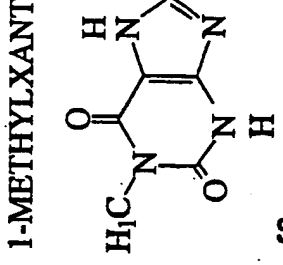
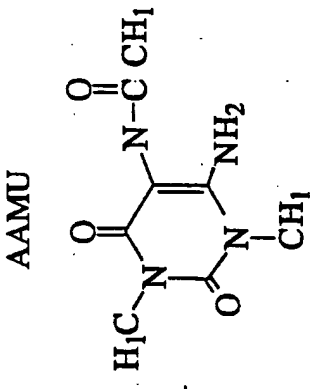
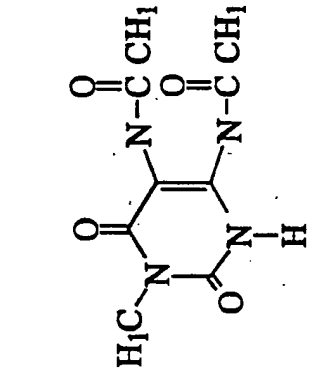


Fig. 2A





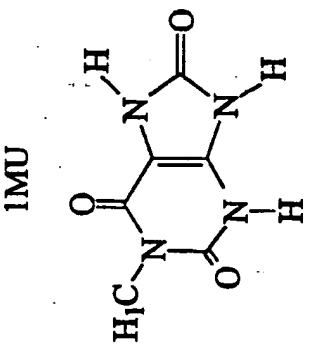
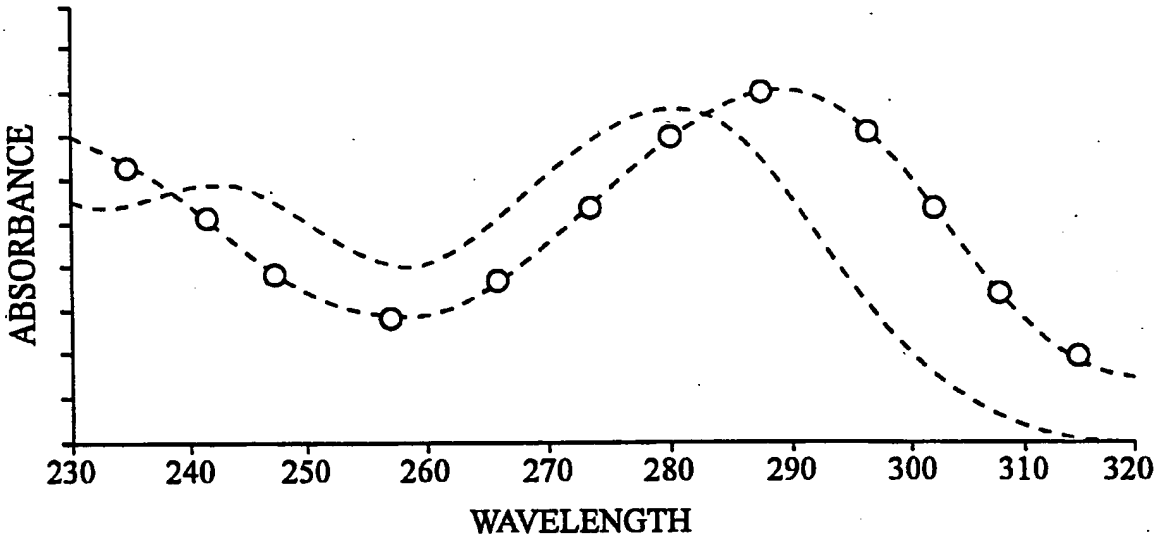


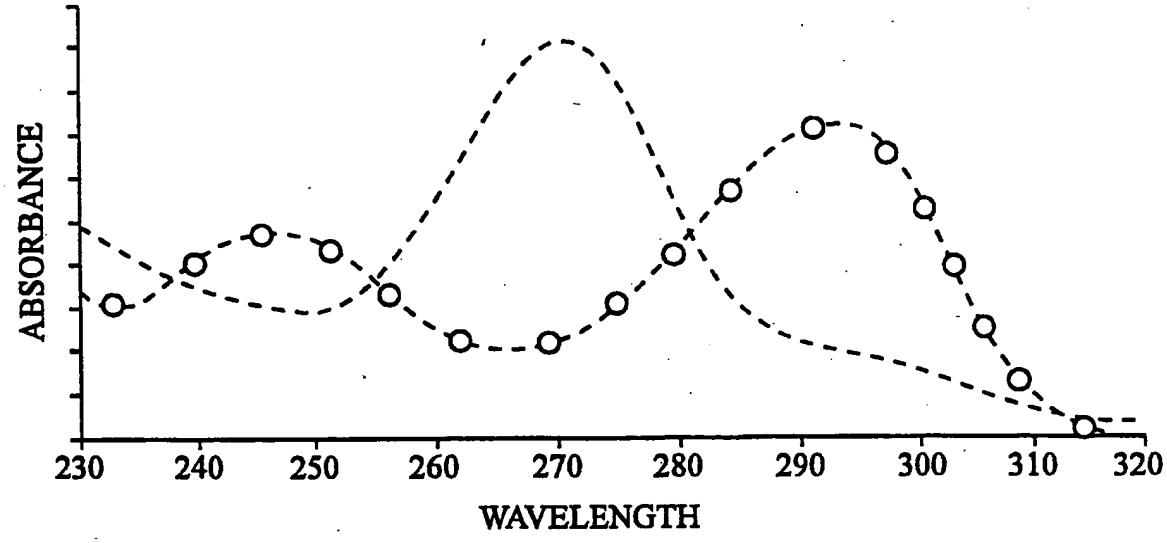
Fig. 2B



--- 1-Methylxanthine wavelength maximum 242,280

-o- 1-Methylxanthine-hexanoic acid wavelength maximum 288

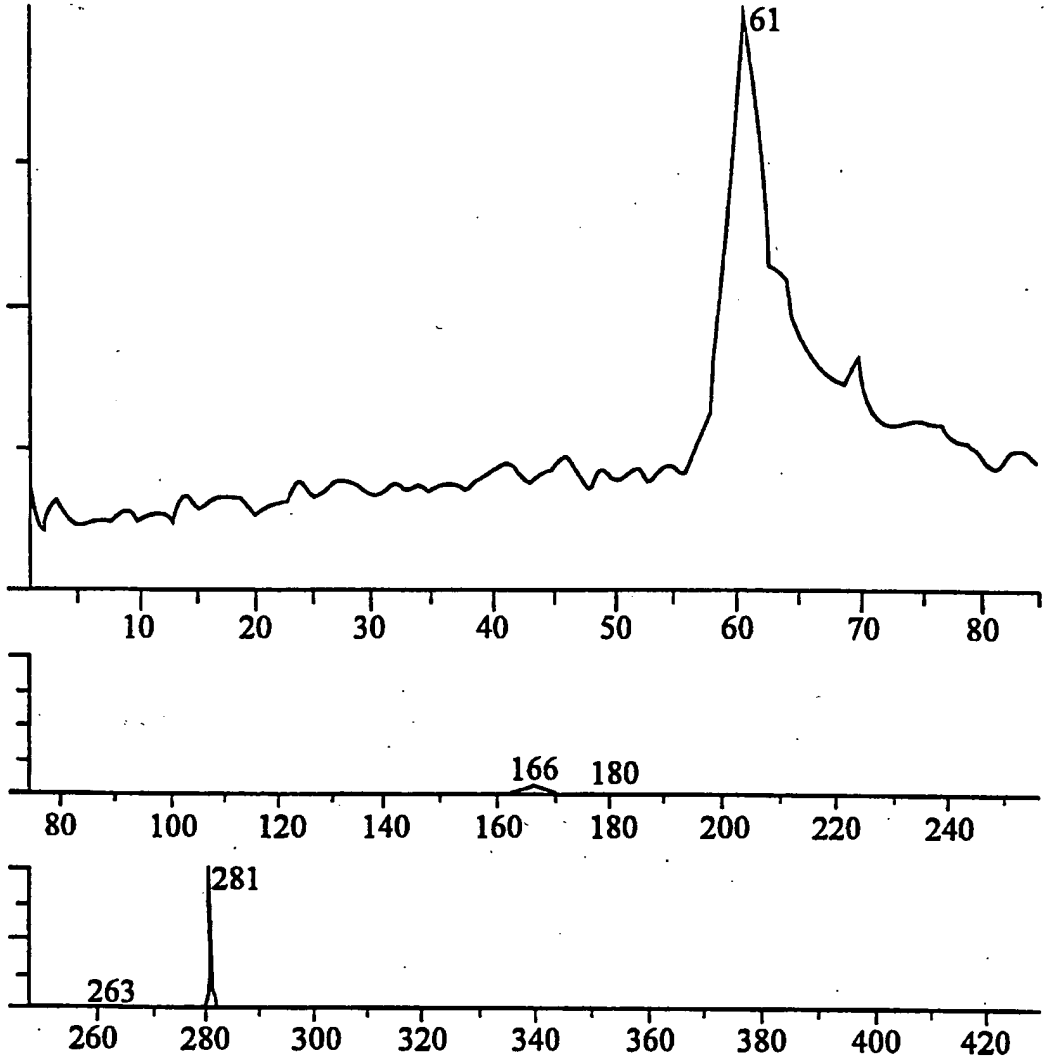
Fig. 3A



-o- 1-Methyluric acid wavelength maximum 236,290

--- 5-amino-6-acetylamino-1-methyluracil wavelength maximum 264

Fig. 3B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166 180
80 100 120 140 160 180 200 220 240
281
263
260 280 300 320 340 360 380 400 420

Fig. 4A

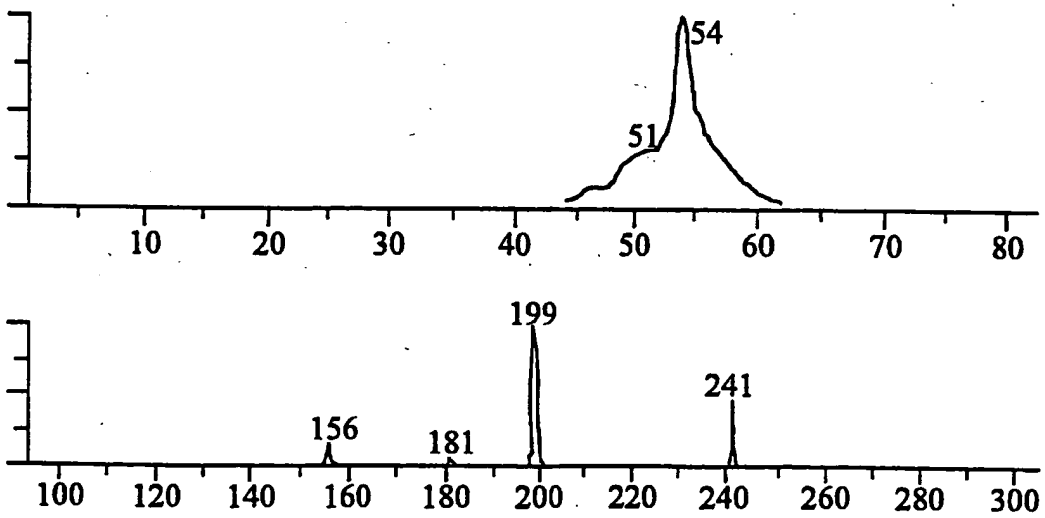
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Fig. 4B

Fig. 5B

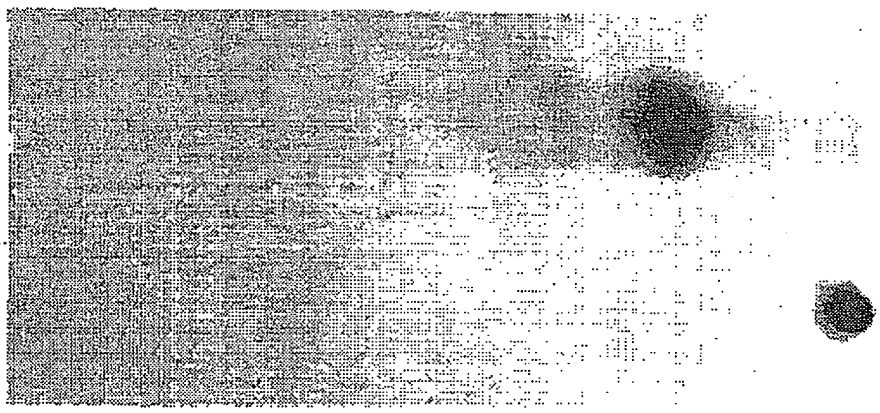
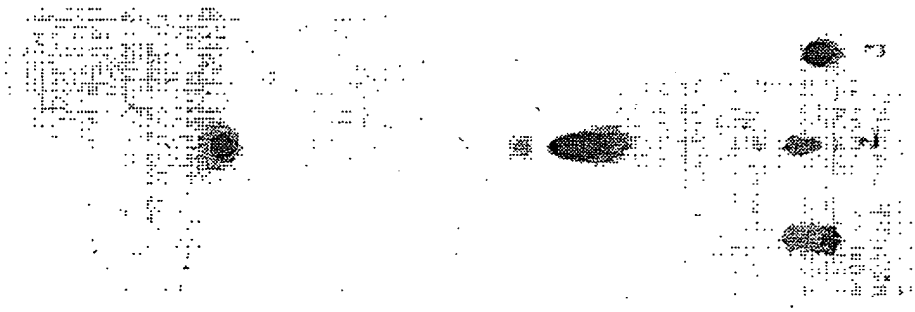
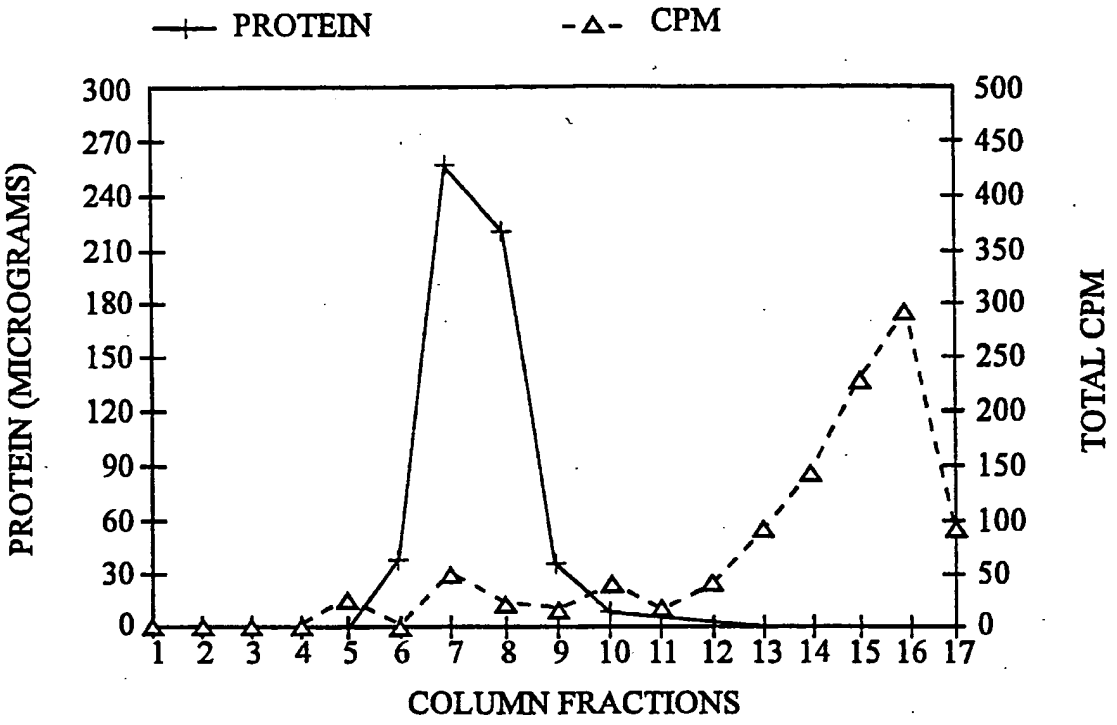


Fig. 5A




PROTEIN
CPM
PROTEIN (MICROGRAMS)
TOTAL CPM
COLUMN FRACTIONS
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120
150
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240
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300
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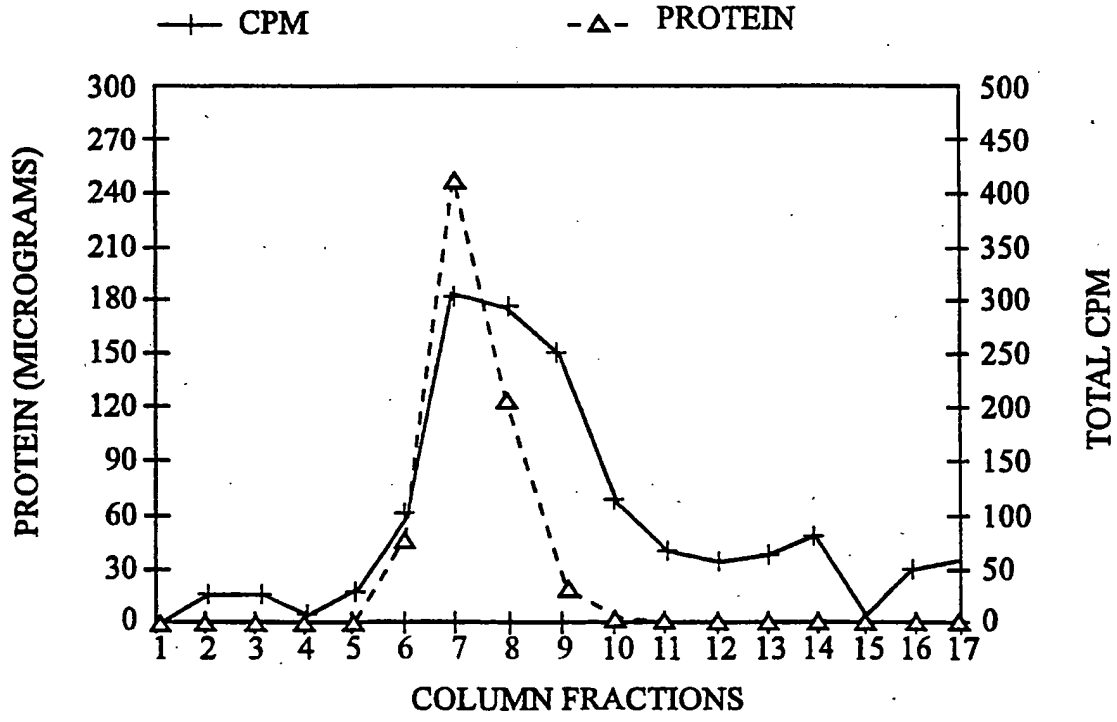
Fig. 6A


CPM
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PROTEIN (MICROGRAMS)
TOTAL CPM
COLUMN FRACTIONS
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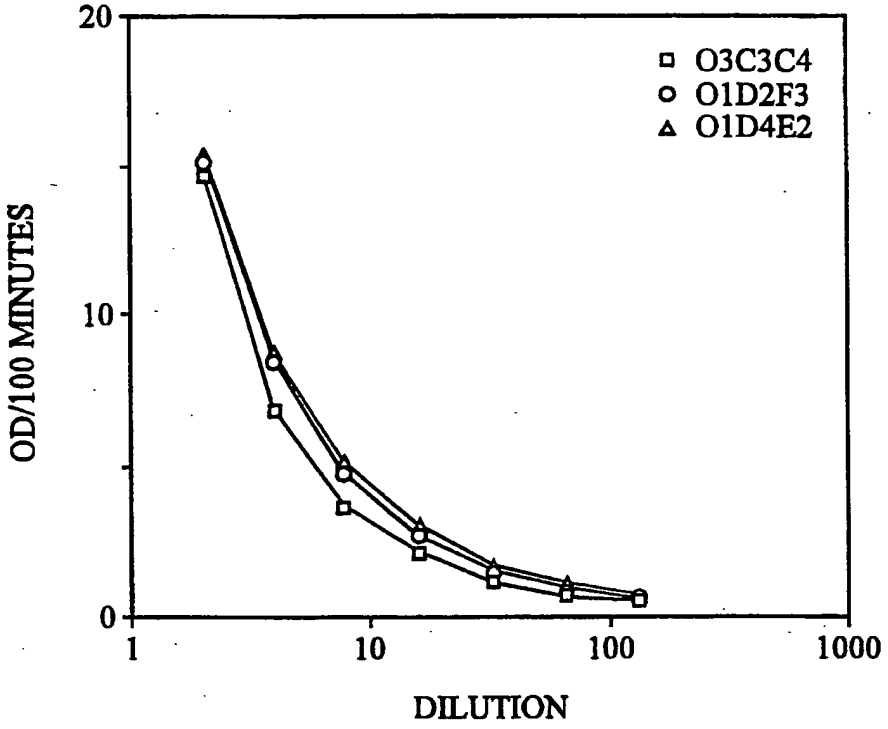
Fig. 6B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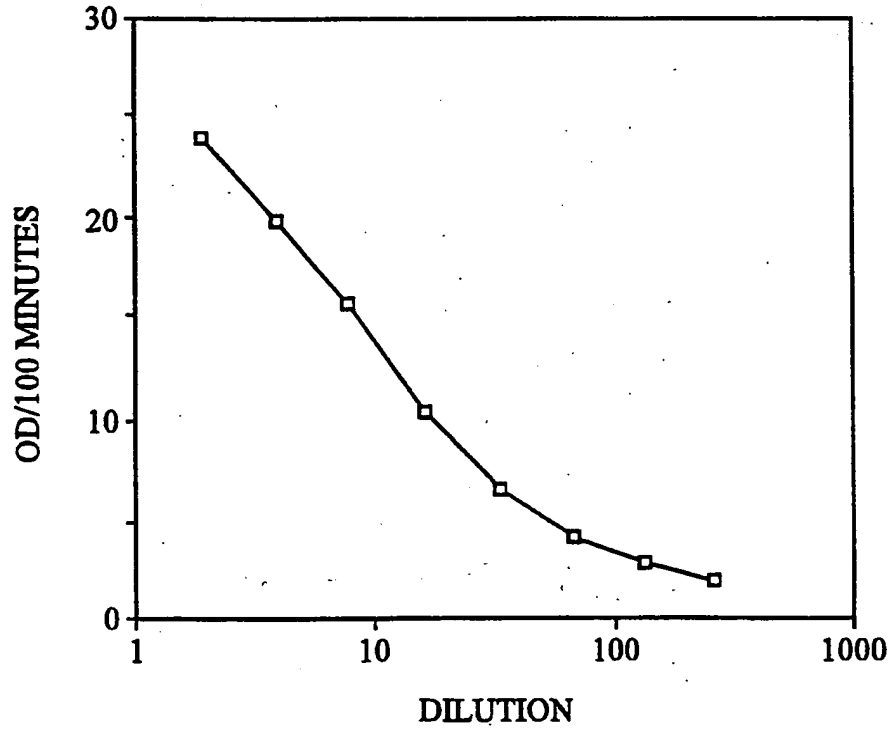
10
100
1000
DILUTION

Fig. 7A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20
10
0
OD/100 MINUTES
1
10
100
1000
DILUTION

Fig. 7B

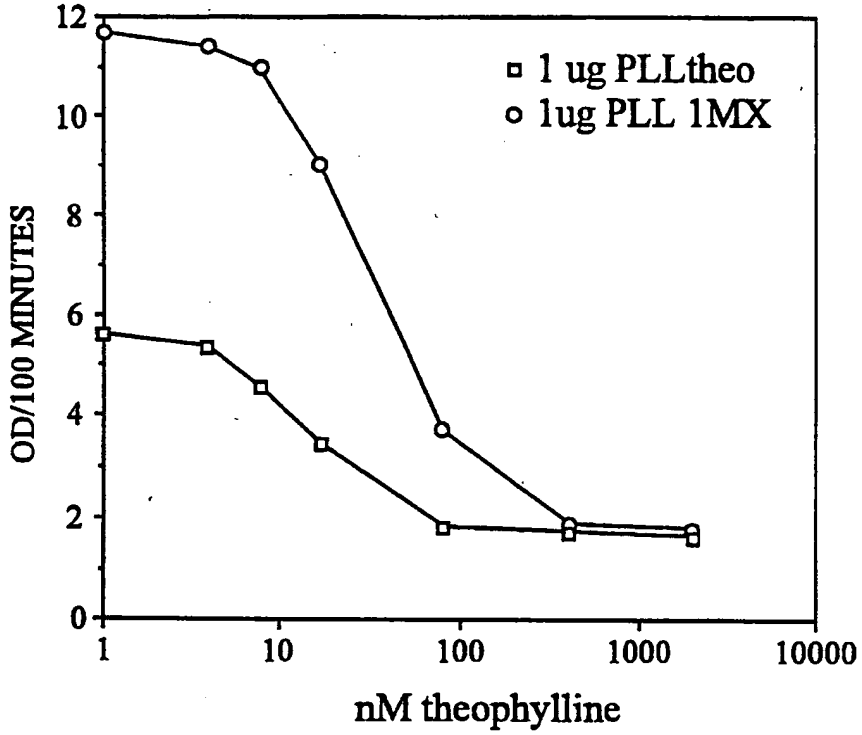
12
10
8
6
4
2
0
OD/100 MINUTES
1 ug PLLtheo
1ug PLL 1MX
1
10
100
1000
10000
nM theophylline

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08882

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 15/28; C12Q 1/00, 1/28; C12N 5/16, 5/18; G01N 33/536, 537, 541
US CL : 530/388.9; 435/7.1, 7.92, 240.27, 810; 436/536, 538, 540, 542
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/388.9; 435/7.1, 7.92, 240.27, 810; 436/536, 538, 540, 542

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Br. J. Clin. Pharmac., Volume 17, issued 1984, D. M. Grant et al, "A simple test for acetylator phenotype using caffeine", pages 459-464, especially pages 459-460.	1, 3-4, 8, 13-30, 37-39
Y	Abstract for Gordon Conference, issued 10 April 1992, D. K. Hammond et al, "Development of an Immunoassay for Caffeine Metabolite, AAMU".	1, 3-4, 8, 13-30, 37-39
Y	Clin Pharmacol Ther, Volume 50, No. 5, Part 1, issued November 1991, W. Kalow et al, "Use of Caffeine Metabolite Ratios to Explore CYP1A2 and Xanthine Oxidase Activities", pages 508-519, especially page 508.	1, 3-4, 8, 13-30, 37-39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
14 OCTOBER 1994	17 NOV 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	CHRISTINA CHAN
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08882

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Clin Pharmacol Ther, Volume 49, No. 6, issued June 1991, B. K. Tang et al, "Caffeine As a Metabolic Probe: Validation of its Use for Acetylator Phenotyping", pages 648-657, especially page 648.	1, 3-4, 8, 13-30, 37-39
Y	H. H. Fedenberg et al., "Basic and Clinical Immunology", published 1976 by LANGE Medical Publications, (Los Altos, California), pages 32-40, especially page 33.	1, 3-4, 8, 13-30, 37-39
Y	Journal of Immunological Methods, Volume 39, issued 1980, J. W. Goding, "Antibody Production by Hybridomas", pages 285-308, see the entire document.	1, 3-4, 8, 13-30, 37-39
Y	D. M. Weir et al., "Handbook of Experimental Immunology", published 1986 by Blackwell Scientific Publications, (U.S.A.), pages 27.1-27.20, see the entire document.	1, 3-4, 8, 13-30, 37-39

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08882

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1, 3-4, 8, 13-30 and 37-39

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08882

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1, 3-4 and 8, drawn to a monoclonal antibody, a hybridoma and an immunoassay method, Classes 530 and 435, Subclasses 388.9 and 7.1+, respectively.
II. Claims 2 and 5-7, drawn to a monoclonal antibody, a hybridoma and an immunoassay method, Classes 530 and 435, Subclasses 388.9 and 7.1+, respectively.
III. Claims 9-12, 18-19, 23-24 and 28-30, drawn to a method for determining an acetylator phenotype of an individual using an acetylizable drug, Class 436, Subclass 540.
IV. Claims 13-30 and 37-39, drawn to a method for estimating a likely degree of sensitivity to an acetylizable therapeutic drug in an individual and a kit, Classes 436 and 435, Subclasses 540 and 810, respectively.
V. Claims 31-36, drawn to a method for assessing risk of toxicity to an acetylizable therapeutic drug in an individual, Class 436, Subclass 540.
VI. Claim 40, drawn to a process of preparing AAMU, Class 544, Subclass 301.
VII. Claims 41-45, drawn to an AAMU conjugate and a process of preparing an AAMU conjugate, Class 530, Subclasses, 350 and 402.

The inventions as grouped are distinct, each from the other, because they represent different inventive endeavors. The monoclonal antibody, hybridoma and the immunoassay method in Group I would not suggest the monoclonal antibody , hybridoma and the immunoassay method in Group II and the AAMU conjugate in Group VII. The method in Group III would not suggest the methods in Groups IV and V. The process of making AAMU in Group VI would not suggest the process of making AAMU conjugate in Group VII. Groups I-VII are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.